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ORIGINAL REPORT

Phase I Study of Quizartinib Administered Daily to Patients With Relapsed or Refractory Acute Myeloid Leukemia Irrespective of FMS-Like Tyrosine Kinase 3–Internal Tandem Duplication Status

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ABSTRACT

Purpose
FMS-like tyrosine kinase 3–internal tandem duplication (FLT3-ITD) mutations in acute myeloid leukemia (AML) are associated with early relapse and poor survival. Quizartinib potently and selectively inhibits FLT3 kinase activity in preclinical AML models.

Patients and Methods
Quizartinib was administered orally at escalating doses of 12 to 450 mg/day to 76 patients (median age, 60 years; range, 23 to 86 years; with a median of three prior therapies [range, 0 to 12 therapies]), enrolled irrespective of FLT3-ITD mutation status in a phase I, first-in-human study in relapsed or refractory AML.

Results
Responses occurred in 23 (30%) of 76 patients, including 10 (13%) complete remissions (CR) of any type (two CRs, three CRs with incomplete platelet recovery [CRp], five CRs with incomplete hematologic recovery [CRi]) and 13 (17%) with partial remissions (PRs). Of 17 FLT3-ITD–positive patients, nine responded (53%; one CR, one CRp, two CRis, five PRs); of 37 FLT3-ITD–negative patients, five responded (14%; two CRps, three PRs); of 22 with FLT3-ITD–indeterminate/not tested status, nine responded (41%; one CR, three CRis, five PRs). Median duration of response was 13.3 weeks; median survival was 14.0 weeks. The most common drug-related adverse events (> 10% incidence) were nausea (16%), prolonged QT interval (12%), vomiting (11%), and dysgeusia (11%); most were ≤ grade 2. The maximum-tolerated dose was 200 mg/day, and the dose-limiting toxicity was grade 3 QT prolongation. FLT3-ITD phosphorylation was completely inhibited in an in vitro plasma inhibitory assay.

Conclusion
Quizartinib has clinical activity in patients with relapsed/refractory AML, particularly those with FLT3-ITD, and is associated with an acceptable toxicity profile.

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INTRODUCTION

Acute myeloid leukemia (AML) is a hematologic malignancy characterized by numerous genetic abnormalities. FMS-like tyrosine kinase 3 (FLT3) is a class III receptor tyrosine kinase (RTK) that is normally only present on progenitor cells, but is expressed on the blasts of most patients with AML.[1] Activating mutations in FLT3 are common and have been identified in approximately 30% of patients with AML.[2] Internal tandem duplications (ITDs) within the FLT3 juxtamembrane region are observed in 20% to 30% of patients with AML, and point mutations are present in the FLT3 activation loop in 5% to 10% of patients with AML.[2,3] These mutations result in constitutive FLT3 activity[4-7] and are associated with early relapse after standard chemotherapy and poor survival.[8-10] In addition, FLT3 promotes cell proliferation and survival through the STAT5, Ras-Erk, and phosphatidylinositide 3 kinase (PI3K) signaling pathways, making FLT3 an important target for AML therapy.[11]

Several FLT3 inhibitors have been explored in the clinic.[12] Unlike first-generation FLT3 inhibitors,[13-15] quizartinib (previously AC220) is selective for FLT3, with at least 10-fold lower affinity for other RTKs (ie, KIT, RET, CSF1R, and platelet-derived growth factor receptor). Quizartinib inhibits both ITD-mutant and wild-type FLT3 in cell line models.[16]

Quizartinib has been evaluated in general toxicity, reproductive toxicity, cardiovascular safety pharmacology, and genotoxicity studies. The principal target organs are lymphoid tissues and bone marrow. In addition, toxicologic effects were observed in the kidney, liver, ovaries, vagina, and testes. In this report, we present the results of a phase 1 study of quizartinib in patients with relapsed or refractory AML enrolled irrespective of FLT3-ITD status.

PATIENTS AND METHODS

Study Design

This phase I open-label, dose-escalation study was conducted at seven centers in the United States and the Republic of Georgia. The primary objectives were to determine the safety, tolerability, and pharmacokinetics (PK) of quizartinib administered daily to patients with relapsed or refractory AML or patients with newly diagnosed AML not eligible for standard induction chemotherapy. Secondary objectives were to determine the pharmacodynamic (PD) parameters of quizartinib and to evaluate antileukemic activity. The protocol was approved by the institutional review boards at all participating centers, and all patients signed an informed consent document according to the Declaration of Helsinki.

Concomitant use of hydroxyurea was allowed for up to 5 days during the first 28 days of this study, up to a maximum dose of 5 g/day. Prior treatment with an FLT3 inhibitor was allowed.

Quizartinib was administered on an empty stomach as an oral solution at a starting dose of 12 mg on an intermittent schedule of 14 days followed by a 14-day rest for each 28-day cycle. The protocol was later amended for the last two study cohorts to have quizartinib administered on a continuous basis in 28-day cycles. Dose escalation used a standard 3+3 design with 50% dose increments. Patients who did not experience a dose-limiting toxicity (DLT) or significant disease progression by day 29, as defined by doubling from baseline of the blast percentage in blood or bone marrow and/or clinical progression, could continue receiving quizartinib. Any nonhematologic toxicity $\geq$ grade 3 considered at least possibly related to quizartinib was considered a DLT. Prolonged myelosuppression, in the setting of less than 5% marrow cellularity on day 57 from start of therapy or later without evidence of leukemia, was also considered as DLT. If a patient experienced a DLT during the first cycle at a dose level, at least three additional patients were enrolled at the same dose. If two or more patients experienced DLT at a given dose level, then accrual was stopped for this cohort and the next lower dose was declared the maximum-tolerated dose (MTD). No DLT was identified up to 450 mg on an intermittent schedule, and continuous dosing (ie, daily administration with no interruptions) was then explored starting at 200 mg/day.

Patients

Patients aged $\geq$ 18 years with histopathologically documented primary or secondary AML (excluding acute promyelocytic leukemia) meeting at least one of the following criteria were enrolled: refractory to one or more cycles of standard chemotherapy, relapsed after one or more cycles of induction chemotherapy, or not a candidate for standard chemotherapy because of age, comorbidity, or other factors. Eastern Cooperative Oncology Group performance status $\leq$ 3 and adequate cardiac, renal, and hepatic function were required.

Exclusion criteria included bone marrow transplantation within 2 months, uncontrolled infection, clinically active CNS leukemia, HIV positivity, hepatitis B or C or other active liver disease, or persistent clinically significant toxicity $\geq$ grade 2 from prior chemotherapy. Patients could not have had major surgery or radiation therapy within 4 weeks before or concurrent with the first dose of quizartinib. Patients with current or prior New York Heart Association functional class 3 or 4 congestive heart failure were excluded unless a screening echocardiogram performed within 3 months demonstrated a left ventricular ejection fraction of $\geq$ 45%.

Tolerability and Safety Assessments

Adverse events (AEs) were graded in accordance with the National Cancer Institute Common Terminology Criteria, version 3.0. Electrocardiograms (ECGs) were obtained at screening, days 1 and 8 (predose and 2 and 4 hours postdose), and days 15, 22, 29, 36, 43, and 57 (predose). Physical examination, vital signs, solicitation of AEs, CBCs, clinical chemistry, and urinalyses were performed at baseline and throughout the study.

Response to Treatment

Patients underwent bone marrow aspirates and/or biopsies at screening and on days 15 and 28. Responses were defined per standard criteria except that participants achieving a complete response (CR) with incomplete hematologic recovery (CRi) or a partial response (PR) were not required to be transfusion independent.[17]

Statistical Analyses

Safety and efficacy data analyses were conducted on all patients receiving at least one dose of quizartinib. Analyses consisted of data summaries of response, AEs, and PK and PD parameters, including area under the curve (AUC), maximum concentration (C_{max}), time to peak concentration (T_{max}), and terminal half-life ($t_{1/2}$). Laboratory parameters were analyzed using descriptive statistics and by evaluating shifts in results between pre- and postdose and examining clinically significant abnormalities. Descriptive statistics of change from baseline values were used to describe vital signs and ECG data. Time-to-event variables, including overall survival and duration of response, were summarized by Kaplan-Meier estimates.

RESULTS

Patient Characteristics

Of 76 patients enrolled, 51 received quizartinib on an intermittent schedule (at daily doses of 12 to 450 mg) and 25 on a continuous schedule (200 to 300 mg daily; Table 1). The median age was 60 years. Sixteen patients (21%) had prior myelodysplastic syndrome. All but three patients (4%, who were $\geq$ 60 years of age) had received prior chemotherapy. Approximately two thirds of patients had received three or more prior lines of therapy, including stem-cell transplantation in 12 (16%). Overall, 30 (39%) of 76 patients were refractory to their first-line AML treatment. A higher percentage of patients in the intermittent dosing groups were refractory to their first AML treatment (23 [45%] of 51) compared with the continuous dosing groups (seven [28%] of 25). FLT3-ITD mutation status was determined at enrollment; 17 patients (22%) were FLT3-ITD positive, 37 (49%) were FLT3-ITD negative, and 22 (29%) were indeterminate/not tested (ind).

Safety and Tolerability

Ten dose levels of quizartinib were investigated using the intermittent schedule (12 to 450 mg) and two in the daily dosing schedule (200 or 300 mg). Quizartinib was generally well tolerated, and approximately half of the AEs observed were not considered related to the study drug. The most frequent treatment-related AEs (observed in $>$ five patients) were $\leq$ grade 2 and included nausea (16%), QT/QTcF prolongation (12%), dysgeusia (11%), and vomiting (11%; Table 2). These symptoms were manageable through treatment interruptions, dose reductions, and medical intervention (eg, antiemetics, antidiarrheal medications). Grade 3 AEs related to quizartinib occurring in more than one patient were prolonged ECG QT interval (four patients [5%]), anemia (three patients [4%]), and fatigue (two patients [3%]). Two treatment-related grade 4 AEs (thrombocytopenia and hypoalbuminemia) were reported each in one patient (1%).

The MTD was 200 mg/day of continuous dosing; no MTD was identified using the intermittent schedule. The DLT was grade 3 QTcF prolongation, occurring in three (38%) of eight patients at 300 mg/day

Table 1. Patient Characteristics

Characteristic	Quizartinib Dose Groups: 12-450 mg ID (n = 51) No.	%	200-300 mg CD (n = 25) No.	%	Total (N = 76) No.	%
Sex						
Male	30	59	16	64	46	61
Female	21	41	9	36	30	40
Age, years						
Mean	57.8		52.4		56.0	
SD	18		15		17	
Median	64.0		52.0		59.5	
Range	23-86		24-74		23-86	
< 60	22	43	16	64	38	50
≥ 60	29	57	9	36	38	50
AML diagnosis						
Primary	39	77	21	84	60	79
Secondary	12	24	4	16	16	21
FLT3-ITD status						
Positive	10	20	7	28	17	22
Negative	26	51	11	44	37	49
Indeterminate/not tested	15	29	7	28	22	29
Previous transplant						
Yes	11	22	4	16	15	20
Type of transplant						
Autologous	3	6	0		3	4
Allogeneic	8	16	4	16	12	16
No. of previous lines of therapy						
0	3	6	0		3	4
1	5	10	1	4	6	8
2	10	20	7	28	17	22
≥ 3	33	65	17	68	50	66
Refractory to first line of therapy						
Yes	23	45	7	28	30	40
No	25	49	17	68	42	55
Unknown	3	6	1	4	4	5

Abbreviations: AML, acute myeloid leukemia; CD, continuous dosing; FLT3-ITD, FMS-like tyrosine kinase 3-internal tandem duplication; ID, intermittent dosing; SD, standard deviation.

Table 2. Incidence of Common (≥ 10%) and All Grade 3 or 4 Related Adverse Events by Preferred Term, Severity, and Dose Group

Preferred Term and Grade	12-135 mg ID (n = 35) No.	%	200-450 mg ID (n = 16) No.	%	200-300 mg CD (n = 25) No.	%	Total (N = 76) No.	%
Nausea								
Total	6	17	3	19	3	12	12	16
Grades 3-4	0		0		0		0	
ECG QT prolonged								
Total	1	3	0		8	32	9	12
Grades 3-4	0		0		4	16	4	5
Dysgeusia								
Total	4	11	1	6	3	12	8	11
Grades 3-4	0		0		0		0	
Vomiting								
Total	3	9	3	19	2	8	8	11
Grades 3-4	1	3	0		0		1	1
Anorexia								
Total	3	9	1	6	1	4	5	7
Grades 3-4	1	3	0		0		1	1
Fatigue								
Total	2	6	1	6	1	4	4	5
Grades 3-4	1	3	0		1	4	2	3
Anemia								
Total	2	6	1	6	0		3	4
Grades 3-4	2	6	1	6	0		3	4
Hypocalcemia								
Total	2	6	0		0		2	3
Grades 3-4	1	3	0		0		1	1
Pyrexia								
Total	2	6	0		0		2	3
Grades 3-4	1	3	0		0		1	1
Eyelid edema								
Total	0		1	6	0		1	1
Grades 3-4	0		1	6	0		1	1
Hypoalbuminemia								
Total	0		1	6	0		1	1
Grades 3-4	0		1	6	0		1	1
Lung infection								
Total	0		1	6	0		1	1
Grades 3-4	0		1	6	0		1	1
Pancytopenia								
Total	1	3	0		0		1	1
Grades 3-4	1	3	0		0		1	1
Photosensitivity reaction								
Total	0		0		1	4	1	1
Grades 3-4	0		0		1	4	1	1
Thrombocytopenia								
Total	0		0		1	4	1	1
Grades 3-4	0		0		1	4	1	1

NOTE. Severity was defined by National Cancer Institute Common Terminology Criteria version 3. There were no grade 5 treatment-related adverse events.

Abbreviations: CD, continuous dosing; ECG, electrocardiogram; ID, intermittent dosing.

of continuous dosing and in four (24%) of 17 patients at 200 mg/day continuous by central ECG analysis (Table 3). Grade 3 QTcF occurred in three of five and one of four females at 300 mg and 200 mg continuous dosing, respectively, and in zero of three and three of 13 males at 300 mg and 200 mg, respectively. With intermittent dosing, one of four (a woman) and zero of six patients treated at 300 mg and 200 mg, respectively, had similar prolongation. The onset of QTcF more than 500 ms (≥ grade 3) was observed as early as 2 hours after the first dose, with all observations occurring by day 8 of the first cycle. In all instances, grade 3 QTcF prolongation was asymptomatic, with no associated arrhythmias, and reversible on quizartinib discontinuation. No clinically significant effect on heart rate, PR (atrioventricular conduction), or QRS interval duration (depolarization) was noted.

The time-averaged mean change from baseline for QTcF duration was dose dependent as reflected by larger increases in QTcF with higher doses and higher incidence of patients in each subsequent dose group with a QTcF interval change from baseline more than 60 ms (Table 3). No other ECG abnormalities were found to be associated with quizartinib, and there were no cases of grade 4 QT prolongation (ie, torsade de pointes).

Table 3. QTcF Changes by Dose and Schedule (Safety Population)

Dose Group	No. of Males and Females in Dose Group		QTcF > 60 ms Change From Baseline n				QTcF > 500 ms n			
			Male		Female		Male		Female	
	Male	Female	No.	%	No.	%	No.	%	No.	%
12-60 mg ID	14	13	1	7	0		0		1	8
90-135 mg ID	7	1	1	14	0		0		0	
200 mg ID	4	2	0		0		0		0	
300 mg ID	0	4	—		1	25	—		1	25
450 mg ID	5*	1	2	40	—		1	20	0	
200 mg CD	13	4	6	46	1	25	3	23	1	25
300 mg CD	3	5	1	33	2	40	0		3	60

*Includes one male participant who is missing baseline QTcF.
Abbreviations: CD, continuous dosing; ID, intermittent dosing.

Pharmacokinetic Profile of Quizartinib and Metabolite AC886

A pharmacologically active metabolite, AC886, was identified in both preclinical species and human plasma.[16,18] Quizartinib was converted to AC886 rapidly, with AC886 detectable 15 minutes after administration of quizartinib. Patients dosed with 200 mg of quizartinib achieved plasma concentrations for quizartinib and AC886 at day 8 (not shown) of approximately 1,000 and 300 ng/mL, respectively, approximately three-fold higher than on day 1 (Appendix Fig A1, online only). Plasma exposure of quizartinib and AC886 (assessed by C_{max} or AUC) on days 1 and 8 increased dose dependently from 12 to 450 mg (Appendix Fig A1, online only; C_{max}). Quizartinib had a long half-life, estimated at more than 1.5 days; the sampling scheme on day 1 did not permit an adequate assessment of the half-life of AC886. The coefficient of variation (CV%) for $AUC_{0\text{-}24}$ at day 8 varied within and between dose cohorts, ranging from 6% to 194%. There was no clear relationship between variability and dose or half-life.

Ex Vivo pFLT3 Plasma Inhibitory Activity Assay

Plasma from quizartinib-treated patients completely suppressed FLT3 phosphorylation in the plasma inhibitory activity (PIA) assay. In cells expressing FLT3-ITD, inhibition was detectable at 18 mg on day 1, and complete inhibition was observed by day 8 (Fig 1A). At the 60-mg dose, complete inhibition was observed at 2 hours postdose on day 1. At 60 mg, but not 18 mg, complete suppression of FLT3-ITD phosphorylation was also noted on day 28, after 14 days off quizartinib (intermittent schedule). Inhibition was also observed in cells expressing wild-type FLT3, although even at 60 mg, detectable pFLT3 was still present at day 8 (Fig 1B).

Pharmacodynamic Assessments

Quizartinib treatment led to decreased pFLT3 (Figs 1C and 2D), pSTAT5, and pKIT levels (not shown). The day 1, 2-hour postdose level of FLT3 phosphorylation compared with baseline phosphorylation in patients who received more than 60 mg of quizartinib decreased for almost all patients, although there was marked variability between individual patients. Median pFLT3 levels were lower for FLT3-ITD–positive patients compared with FLT3-ITD–negative patients at both 2 and 24 hours after quizartinib ($P < .05$, Mann-Whitney U test) on day 1.

Response to Treatment

Responses were observed in 23 (30%) of 76 patients (Table 4), with 10 patients (13%) having a CR of any type (two CRs, three CRs with incomplete platelet recovery [CRp], five CRis) and 13 (17%) a PR. Responses were observed at doses as low as 18 mg/day (intermittent dosing). Nine (53%) of 17 FLT3-ITD–positive patients responded (95% CI, 28% to 77%]; one CR, one CRp, two CRis, five PRs) compared with five (14%) of 37 FLT3-ITD–negative patients (95% CI, 5% to 29%; two CRps, three PRs) and nine (41%) of 22 with FLT3-ITD(ind) status (95% CI, 21% to 64%; one CR, three CRis, five PRs). Median response duration was 13 weeks (95% CI, 11 to 29 weeks): 10 weeks for FLT3-ITD–positive patients (95% CI, 4 to 29 weeks), 24 weeks for FLT3-ITD–negative patients (95% CI, 11 weeks to not reached), and 12 weeks for FLT3-ITD(ind) patients (95% CI, 8 to 53 weeks).

Survival

Median overall survival (Fig 2) was 14 weeks (95% CI, 11 to 19 weeks): 18 weeks for FLT3-ITD–positive patients (95% CI, 11 to 27 weeks), 10 weeks for FLT3-ITD–negative patients (95% CI, 6 to 14 weeks), and 19 weeks for FLT3-ITD(ind) patients (95% CI, 14 to 21 weeks). Median survival was 35 weeks for patients with a CRc (CR + CRp + CRi) and 10 weeks for nonresponders in the intent-to-treat population (Fig 2). When survival was analyzed only for patients who survived at least 28 days, median survival was 35 weeks for patients with CRc and 13 weeks for nonresponders (not shown).

DISCUSSION

This first-in-human phase I study of the FLT3 inhibitor quizartinib in patients with relapsed or refractory AML demonstrated that quizartinib was well tolerated at daily doses ≤ 200 mg/day and provided encouraging evidence of single-agent activity.

In this study, responses were observed for patients who had been heavily pretreated with a median of three prior treatment regimens, and 40% were refractory to their first line of therapy. The overall response rate was 30%, with 13% having a CR of any type. Responses to quizartinib were rapid, with the majority occurring after the first treatment cycle.

Quizartinib yielded a higher overall response rate in patients who were FLT3-ITD positive (nine of 17, 53%) compared with patients

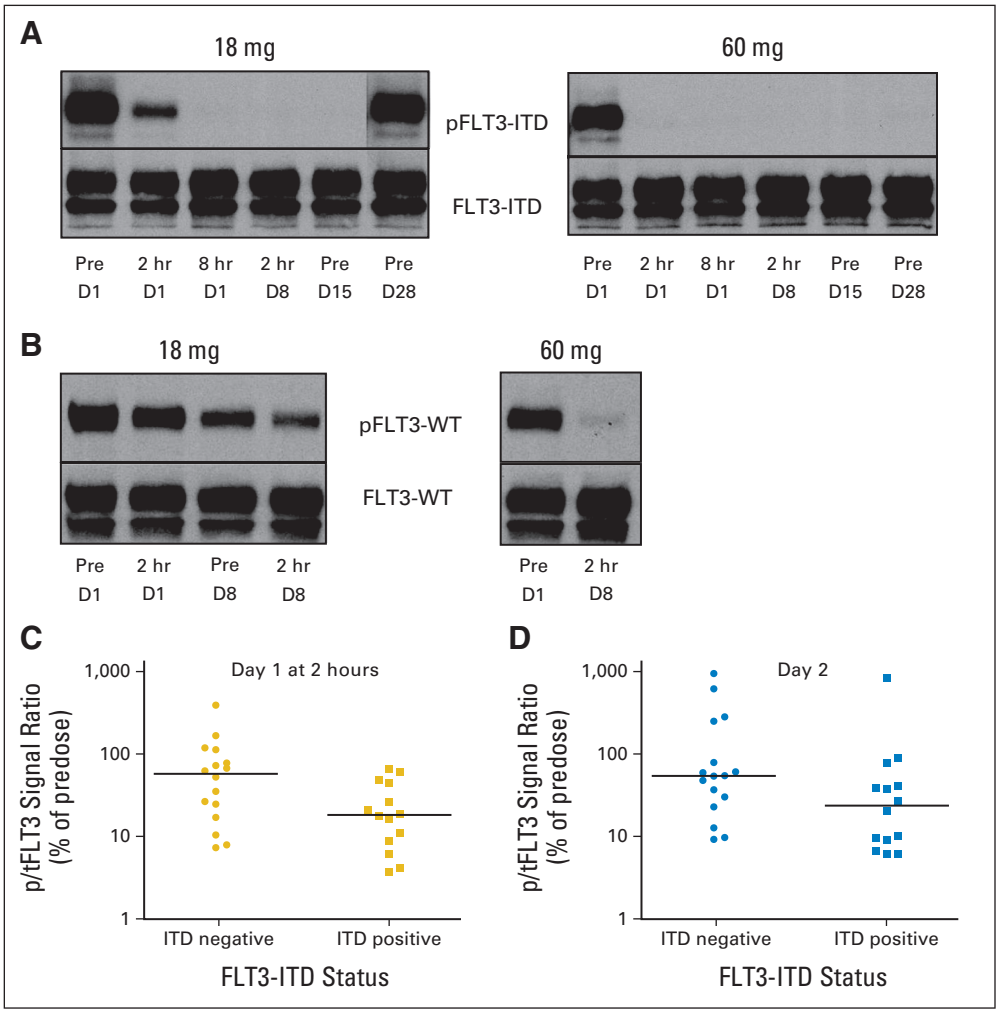

Fig1. Inhibition of FMS-like tyrosine kinase 3 (FLT3) phosphorylation by quizartinib. (A, B) Ex vivo plasma inhibitory assay for FLT3. Results from representative patients are shown for inhibition of (A) FLT3–internal tandem duplication (ITD) and for (B) wild-type FLT3 for samples taken predose (Pre) and postdose on days shown. (C, D) Effect of quizartinib on pFLT3 in peripheral blood at 2 hours (day 1 [D1]) and 24 hours (day 2) postdose. The y-axis indicates the ratio of pFLT3 to total FLT3 expressed as a percentage of baseline. On day 1, median pFLT3 for FLT3-ITD–positive patients was 18.2% versus 57.4% for FLT3-ITD–negative patients ($P = .021$). On day 2, median pFLT3 for FLT3-ITD–positive patients was 23.7%% versus 54.3% for FLT3-ITD–negative patients ($P = .0586$). Only patients treated with more than 60 mg quizartinib were included. p/tFLT3 = phosphorylated FLT3/total FLT3.

with FLT3-ITD–negative status (five of 37, 14%) and those in whom FLT3-ITD status was indeterminate/not tested (nine of 22, 41%). Median duration of response was shorter for FLT3-ITD–positive patients than for FLT3-ITD–negative ones, 10 weeks versus 24 weeks, respectively. The short median duration of remission is likely a reflection of the complex molecular milieu of acute leukemias. Inhibition of one molecular event, regardless of how meaningful biologically or prognostically it may be, is likely insufficient for disease eradication. In addition, secondary escape mutations have been reported to occur.[19] The mechanism of quizartinib activity in FLT3-ITD–negative patients is not clear. However, both wild-type FLT3 and c-KIT are commonly overexpressed in AML,[20,21] and c-KIT mutations are found in approximately 6% of patients with AML.[22] Quizartinib inhibits wild-type FLT3 and c-KIT but with an approximately 4- and 10-fold higher half-maximal inhibitory concentration than mutant FLT3-ITD, respectively, and this may be the basis for the activity seen in FLT3-ITD–negative AML.[16]

The most frequent grade 3 treatment-related AEs were QT/QTcF prolongation, anemia, and fatigue. There were two quizartinib-related grade 4 AEs (one thrombocytopenia and one hypoalbuminemia), although a relationship with AML and/or comorbidities cannot be ruled out. There were no quizartinib-related deaths; all deaths during and after the study were either associated with the disease and/or preexisting comorbidities. This is in contrast to other AML therapies, including standard induction chemotherapy, which are associated with significant 30-day treatment-related mortality.[23]

QTc prolongation is observed with several kinase inhibitors, including nilotinib, dasatinib, sunitinib, and vandetanib.[24-26] Cardiac safety data indicated that quizartinib had a marked effect on cardiac repolarization at doses $\geq$ 200 mg/day. Although there was a dose- and sex-dependent effect on QTcF, with more significant prolongation in female patients, it should be noted that this study did not exclude high-risk cardiac patients, including those with abnormal baseline QTcF prolongation, nor did it exclude the administration of QT/QTc-prolonging concomitant medications, and patients sometimes had low serum potassium and/or magnesium levels. In all instances, grade 3 QTcF prolongation was asymptomatic, with no associated arrhythmias, and reversible. No other ECG abnormalities were found to be associated with quizartinib, and there were no cases of torsade de pointes.

The results from the PIA assays are consistent with preclinical observations, suggesting that quizartinib is the most potent FLT3 inhibitor tested to date. The high degree of in vivo inhibition observed with quizartinib is well beyond the inhibition achieved with any prior FLT3 inhibitor (lestaurtinib, midostaurin, KW-2449, sorafenib) assessed with the PIA assay.[14,27-29] The PIA results suggest that robust

Table 4. Response by Quizartinib Dose and Schedule and FLT3 Status

Dose	FLT3-ITD Positive (n = 17) No.	FLT3-ITD Positive (n = 17) Responses	FLT3-ITD Negative (n = 37) No.	FLT3-ITD Negative (n = 37) Responses	FLT3-ITD (ind) (n = 22) No.	FLT3-ITD (ind) (n = 22) Responses
12 mg ID, n = 3	0		1	0	2	0
18 mg ID, n = 8	0		4	1 PR	4	0
27 mg ID, n = 6	0		3	0	3	2 PR
40 mg ID, n = 5	0		4	1 CRp, 1 PR	1	0
60 mg ID, n = 5	1	1 CRi	2	0	2	1 PR
90 mg ID, n = 3	2	0	1	1 PR	0	
135 mg ID, n = 5	2	0	2	0	1	1 PR
200 mg ID, n = 6	3	1 PR	3	1 CRp	0	
300 mg ID, n = 4	1	1 PR	2	0	1	1 CRi
450 mg ID, n = 6	1	1 PR	4	0	1	1 PR
200 mg CD, n = 17	6	1 CR, 1 CRp, 1 CRi, 1 PR	7	0	4	1 CR, 1 CRi
300 mg CD, n = 8	1	1 PR	4	0	3	1 CRi

Abbreviations: CD, continuous dosing; CR, complete remission; CRp, CR with incomplete platelet recovery; CRi, CR with incomplete neutrophil recovery; FLT3-ITD, FMS-like tyrosine kinase 3-internal tandem duplication; ind, indeterminate/not tested; ID, intermittent dosing; PR, partial remission.

inhibition of pFLT3 is achievable at steady-state with doses as low as 18 mg, at which level a clinical response was observed. Remarkably, at 60 mg, intermittent dosing FLT3-ITD phosphorylation was completely suppressed at 28 days after 14 days off quizartinib. This demonstrates that quizartinib has the potential for continuous suppression of FLT3 phosphorylation in vivo at dose levels that are well tolerated. Complete inhibition of FLT3 may be important for clinical activity to overcome upregulation of FLT3 ligand, which is a possible mechanism of resistance to therapy.[30]

PK analysis indicated that drug exposure was dose dependent. Quizartinib was rapidly converted to its metabolite (AC886), which has a similar kinase selectivity profile and is as potent as quizartinib.[16,18] Half-maximal inhibitory concentration values in FLT3-ITD–expressing cell lines were 1.1 nmol/L for quizartinib and 0.3 nmol/L for AC886. Both compounds display a high degree of selectivity for FLT3 in an interleukin-3 rescue assay.[18] The PK profile for quizartinib and AC886 were characterized by a rapid rise to C_{max} followed by a prolonged terminal phase. Both quizartinib and AC886 had relatively long half-lives, estimated at more than 1.5 days for quizartinib and longer for AC886, leading to drug accumulation over the 8-day PK assessment period. A long half-life for quizartinib and its active metabolite explains the prolonged activity seen in the in vitro PIA assay up to 14 days after cessation of dosing. Importantly, the bioanalytical assay used for assessment of quizartinib and AC886 plasma concentrations was exploratory, and reproducibility was not confirmed; therefore the PK data should be interpreted cautiously.

The encouraging efficacy results in both FLT3-ITD–positive and FLT3 ITD–negative patients and an acceptable safety profile in this high-risk population support continued clinical evaluation of quizartinib. The improved activity of quizartinib in vivo compared with other FLT3 inhibitors is likely due to its high potency and degree of selectivity and the favorable pharmacokinetics with long half-life likely resulting in continuous suppression of FLT3-ITD signaling at doses well below the MTD. Additional phase II studies are being conducted in both FLT3-ITD–positive and FLT3-ITD–negative AML to confirm clinical efficacy and examine lower doses to reduce the incidence of QT prolongation and determine the optimal dose.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were

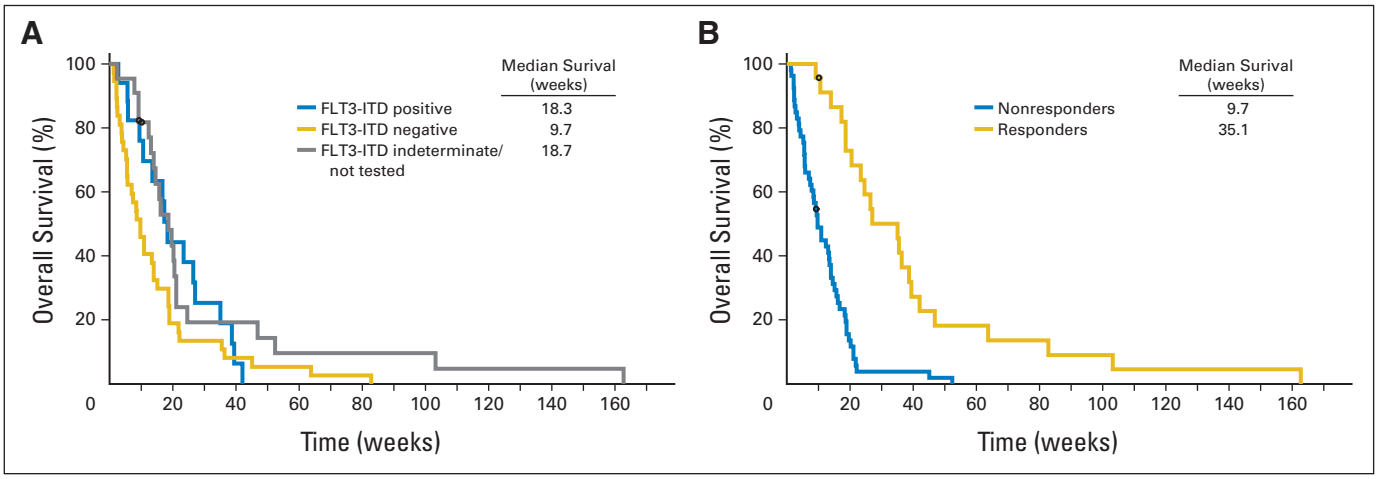

Fig 2. Kaplan-Meier curve of overall survival (A) by FMS-like tyrosine kinase 3–internal tandem duplication (FLT3-ITD) status and (B) by response. (A) Median overall survival was 14 weeks (95% CI, 11 to 19 weeks): 18 weeks for FLT3-ITD–positive patients (95% CI, 11 to 27 weeks), 10 weeks for FLT3-ITD–negative patients (95% CI, 6 to 14 weeks), and 19 weeks for FLT3-ITD indeterminate/not tested patients (95% CI, 14 to 21 weeks). Two participants were lost to follow-up and are censored for overall survival: one at 9 weeks in the FLT3-ITD–positive group and one at 10 weeks in the FLT3-ITD(ind) group. (B) Overall survival for the intent-to-treat population after the initial dose of quizartinib divided into those who achieved a best response of complete remission (CR), CR with incomplete platelet recovery, CR with incomplete neutrophil recovery, or partial remission compared with nonresponders. Two participants were lost to follow-up and are censored for overall survival: one at 9 weeks in the nonresponder group and one at 10 weeks in the CR group.

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Appendix

Evaluation of QTcF Prolongation

ECGs were analyzed by the investigators and subsequently reread at a central ECG laboratory (eRT, Philadelphia, PA). The QTc interval was corrected by the Fridericia method (QTcF).

Pharmacokinetic Assessments

Plasma samples were collected for pharmacokinetic analysis of quizartinib and its active metabolite, AC886, on days 1 and 8 (predose and 0.25, 0.5, 1, 2, 4, 6, 9, and 24 hours postdose) and day 15 (approximately 24 hours after the final dose). The bioanalytical assay used for assessment of quizartinib and AC886 plasma concentrations was exploratory, and reproducibility was not confirmed.

FLT3-ITD Mutation Status

FMS-like tyrosine kinase 3–internal tandem duplication (FLT3-ITD) genotyping was performed on baseline samples for all available patient peripheral blood samples. After DNA isolation, a standard polymerase chain reaction–based genotype analysis was conducted by a central genotyping lab (Murphy KM, et al: J Mol Diagn 5:96-102, 2003). To be able to determine FLT3-ITD mutation status, participants had to have at least 6% blasts in the peripheral blood specimen tested and a 1% ITD to wild-type ratio was used as the cutoff for positive. Patients were eligible for the study regardless of their FLT3-ITD status.

Ex Vivo Plasma Inhibitory Activity Assay for FLT3

Patient plasma samples were collected predose and 2 hours postdose on days 1, 8, 15, and/or 28, and a subset was analyzed in the ex vivo plasma inhibitory activity assay to measure FLT3 phosphorylation.[27] The TF-ITD cell line was used for FLT3-ITD activity,[27] and the SEMK2 cell line was used for wild-type FLT3 activity (Greil J, et al: Br J Haematol 86:275-283, 1994). Cells were lysed, the extract was clarified by centrifugation at 14,000 rpm, and the supernatant was assayed for protein. Anti-FLT3 antibody was added to the extract for overnight incubation, and protein A-Sepharose was added for 2 hours. After sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transfer to Immobilon membranes, immunoblotting was performed with antiphosphotyrosine antibody to detect phosphorylated (p)FLT3. Blots were stripped and reprobed with anti-FLT3 antibody to measure total FLT3. Proteins were visualized using chemiluminescence and scanned using a Bio-Rad GS800 densitometer.

Pharmacodynamic Assessments

Whole-blood samples collected pre- and 2 hours postdose were lysed in lysis buffer for 30 minutes and clarified by centrifugation at 14,000 rpm at 4°C. Cleared lysates were tested for total and pFLT3 using a chemiluminescent sandwich enzyme-linked immunosorbent assay (MesoScale Diagnostics, Rockville, MD). Lysates were incubated overnight at 4°C. The resulting signal for pFLT3 was normalized to total FLT3, and the percentage pFLT3 postdose was calculated relative to the predose normalized pFLT3.

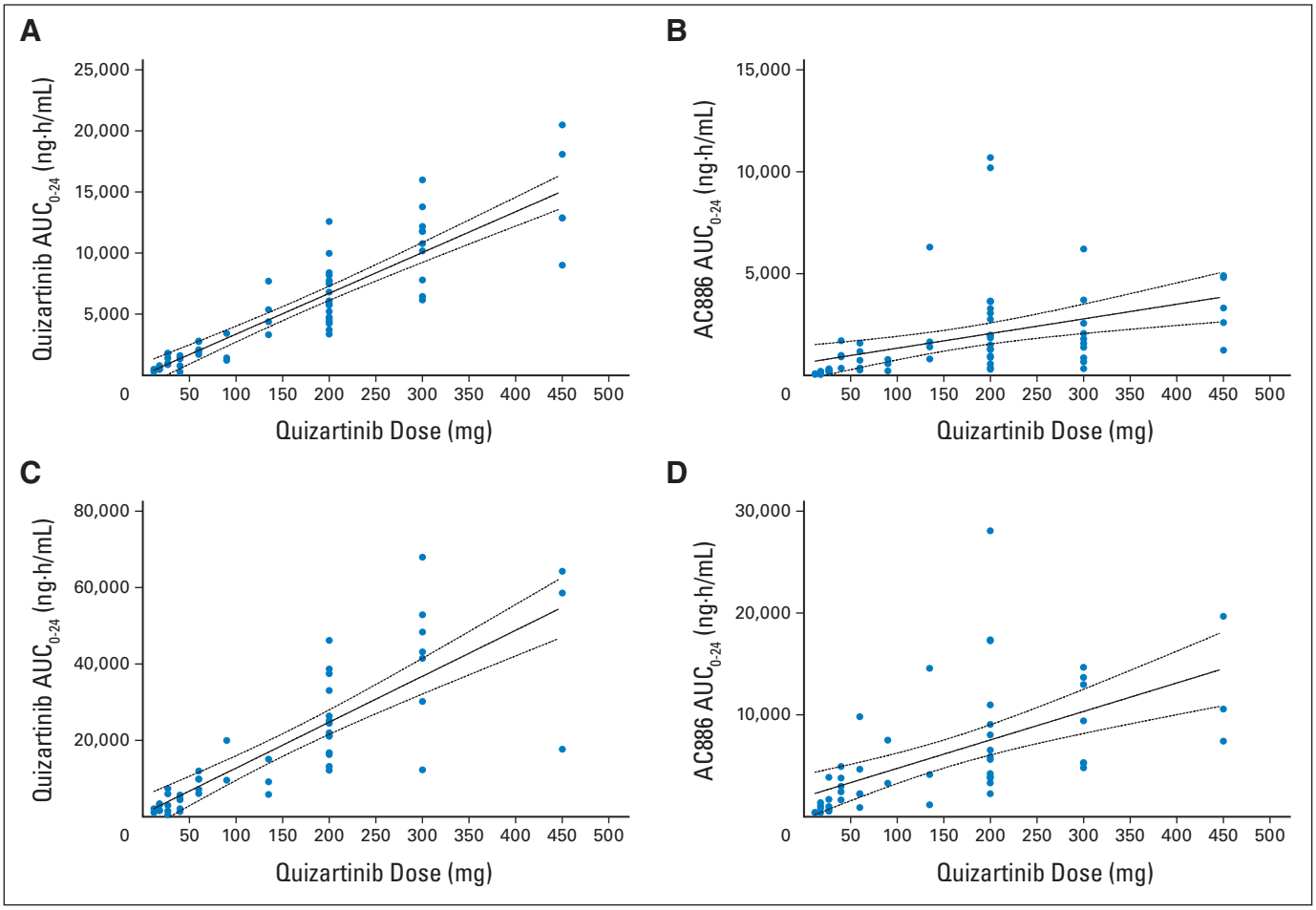

Fig A1. Pharmacokinetics of quizartinib and AC886. (A) Area under the concentration-time curve from 0 to 24 hours ($AUC_{(0-24)}$) of quizartinib by dose group, day 1. (B) $AUC_{(0-24)}$ of AC886 by quizartinib dose group, day 1. (C) $AUC_{(0-24)}$ of quizartinib by dose group, day 8. (D) $AUC_{(0-24)}$ of AC886 by quizartinib dose group, day 8.